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DATA EVALUATION REPORT

STUDY TYPE: 82-4 Subacute (28-Day) Inhalation Toxicity (Rat; submitted under 81-3, Acute Inhalation Toxicity)

TOX. CHEM NO: 603 PC NO.: 056301

MRID NO.: 425387-01

TEST MATERIAL: Paranitrophenol, technical

SYNONYMS/CAS NO.: 4-nitrophenol/000100-02-7

STUDY NUMBER: HLA Study No. 241-139

SPONSOR: U.S. Army Aviation and Troop Command, Natick Research, Development and Engineering Center, Natick, MA 01760-5020

TESTING FACILITY: Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, VA 22182

TITLE OF REPORT: Subacute Dust Inhalation Toxicity Study in Rats - p-Nitrophenol

AUTHOR: William B. Coate, PH.D.

REPORT ISSUED: September 21, 1992 (reformatted study completed 8-16-83)

CONCLUSION:

Doses administered: 0, 0.001, 0.005 or 0.03 mg/L (0, 1, 5 or 30 mg/m^3) particulate paranitrophenol, by inhalation to male and female Sprague-Dawley rats for a total of 20 exposures (6 hr/day, 5 days/week, 4 weeks).

No mortality or significant treatment-related systemic toxicity was observed in any treatment group. Low incidence of keratitis sicca in high dose animals may have been due to local irritation by the test compound dust.

NOEL $\geq$ 0.03 mg/L, males and females

Core Classification: Unacceptable for 81-3 (not upgradable); Supplementary for 82-4 (not upgradable).

This study did not satisfy guidelines for acute inhalation

toxicity (Guideline #81-3) and is not considered acceptable for regulatory purposes for the following reasons: purity of test compound not identified, particle sizes (MMAD) > 4 μM; highest dose did not produce significant systemic toxicity and was below limit dose of 5 mg/L for acute inhalation studies; study not designed to determine single-dose LD_{50}. Because of these study deficiencies, Tox. Category I would be assigned by default.

Signed Quality Assurance and Good Laboratory Practice Statements were present. The study was conducted according to FDA GLP regulations issued on December 22, 1978 but the Sponsor included a statement that they did not know whether the study was conducted according to 40 CFR Part 160.

MATERIALS:

1. Test compound: Paranitrophenol, technical. Description: yellow powder (micronized). Lot #: AC-PN-09004 (Monsanto). Purity - not given in report (on file with sponsor).

2. Test animals: Species: Rat. Strain: Sprague-Dawley. Age: Approx. 6 weeks at study start. Weight: 170 - 233 g (male); 139 - 175 g (female), at start of study. Source: Charles River, Kingston, NY. Quarantine period was 14 days.

3. Animal Care: Housing: individually in stainless steel wire mesh cages prior to exposures; in exposure chambers between exposures. Temperature (between exposures): 70 - 80°C. Humidity (between exposures): 37 - 73%. HEPA-filtered airflow: 167 L/min. Light: 12 hr light/12 hr dark. Food: Purina Rodent Chow #5002. Water: Tap. Both administered ad libitum (prior to and between exposures only).

STUDY DESIGN:

Animal Assignment: Male and female Sprague-Dawley rats were randomly assigned to the following test groups:

TABLE 1: ANIMAL ASSIGNMENT

Test Group	Dose Level (mg/M^3)	Number Assigned
Control	0.0	15
Low Dose	1.0	15
Mid Dose	5.0	15
High Dose	30.0	15

Each dose group was split into 3 squads of 5 animals each, which were initiated and eventually sacrificed on 3 successive

days. Animals were exposed over the course of 28 days for 5 days/week, 6 hrs/day (total of 20 days exposure). On Day 29 all animals were sacrificed. Food and water were provided between exposures but not during.

Exposure Chamber/Aerosol Generation: Whole-body exposure, 1000 l chambers made of glass and stainless-steel were used. Cages were arranged in a single tier in the chambers. The chambers were supplied with test atmosphere by mixing air with paranitrophenol using a Wright dust-feed mechanism connected to the top of each chamber. Hydraulic pressure was used to pack the compound in the dust-feed cups and amount of test material added throughout the study was recorded. Control animals were placed in chambers receiving only filtered air.

Analysis of Test Atmosphere: Nominal concentrations were determined whenever dust feed cups were refilled. Gravimetric dust concentration distribution was determined for each exposure level by measuring under exposure conditions identical to those used in the study and with 3 rats in each chamber. Samples were collected on Gelman DM-450 filters from a probe which sampled the chamber atmospheres at a rate of 5.0 L/min and gravimetric concentrations calculated according to the duration of sampling (60 min, Group 2, 30 min, Group 3 and 15 min, Group 4).

Results - Dust cups were refilled with micronized paranitrophenol 2 times for Group 2 and 7 times for Groups 3 and 4. The mean nominal concentrations were 4.44 ± 1.25 mg/M^3, 20.67 ± 1.13 mg/M^3 and 105.37 ± 2.15 mg/M^3 for Groups 2, 3 and 4, respectively.

Analytical test atmosphere concentration measurements taken from different parts of the exposure chamber before initiation of study are shown below in Table 2:

TABLE 2: TEST ATMOSPHERE CONCENTRATIONS OF P-NITROPHENOL[1]

Sample Position:	Group 2 (1 mg/M^3)	Group 3 (5 mg/M^3)	Group 4 (30 mg/M^3)
Right front	1.13	3.80	32.27
Right rear	1.40	4.07	34.13
Left front	1.23	4.67	35.87
Left rear	1.23	3.93	32.13
Center (Standard Probe)	1.07	3.93	30.40

1 Data taken from table on p. 19 of study report

Concentrations measured in different parts of the chambers showed some variability, particularly at low concentrations. Concentrations of paranitrophenol during exposure were all within

acceptable range of target concentration (mean values for measurements during Weeks 1 - 4 were 9%, 5.4% and -2.7% for Groups 2, 3 and 4, respectively). Variability of individual weekly measurements did not exceed 15% and was usually less than 10%.

Particle size was determined once per week using an Anderson 8-stage cascade impactor with preseparator and backup glass fiber filter. Mass median aerodynamic diameter (MMAD) was calculated from samples collected at 28.4 L/min (Group 2 for 300 min, Group 3 for 120 min and Group 4 for 60 min).

Results - Particle size analysis is shown below in Table 3:

TABLE 3: PARTICLE SIZE ANALYSIS (MMAD)[1]

Exposure level	1 mg/M^3	5 mg/M^3	30 mg/M^3
Week 1	6.2	6.0	6.5
Week 2	5.4	3.5[2]	6.8
Week 3	6.0	3.9[2]	6.9
Week 4	5.5	6.0	6.6
Week 4, Squads 2 and 3	5.9	6.6	6.8

1 Data taken from Table 2 of study
2 Sampling system suspected of leaking for these samplings

All MMAD's measured during the course of this study were larger than 5μm with the exception of the measurements taken with the leaky system.

Chamber temperature and humidity were monitored throughout exposure. There was no mention in the study report of monitoring the oxygen concentration in the chamber.

Results - Temperature measurements ranged from 74° - 81°F and humidity between 35 - 75% during exposures.

Statistical Analyses: Box's test for homogeneity of variances, followed by one-way ANOVA, was performed on body weight, body weight gain, clinical pathology and absolute/relative organ weight data. Where variances were homogeneous, rank transformation of data was performed, then Box's test and ANOVA again. Where ANOVA was significant, Dunnett's t-test was used to compare control and treated groups. Statistical significance was identified at the 5% probability level (two-tailed).

METHODS AND RESULTS:

Mortality: No animals died during exposure or the 2-week

observation period.

Clinical Observations: Animals were observed twice each day for clinical signs of toxicity.

Results - There were no apparent treatment-related clinical signs noted during this study. At Weeks 3 and 4, four control females, 2 low-dose males and 3 low-dose females had opaque left eyes. This may have been related to trauma from the orbital sinus bleeding procedure. Almost all treated animals showed yellow tinge on the fur during the study which was due to the yellow-colored dust.

Ophthalmologic Exam: Examinations were performed just prior to initiation of treatment and at the end of Week 4 prior to terminal sacrifice. Gross eye examination and indirect ophthalmoscopy of the cornea, anterior chambers, lens, vitreous humor, retina and optic disc were performed.

Results - The study authors noted no abnormal observations prior to initiation of exposure. Incidence of various types of cataracts and keratitis sicca identified on Day 20 are shown below in Table 4:

TABLE 4: OPHTHALMOLOGIC EFFECTS AT TERMINATION OF EXPOSURE[1]

	0 mg/M^3		1 mg/M^3		5 mg/M^3		30 mg/M^3	
N = 5, all squads	♂	♀	♂	♀	♂	♀	♂	♀
SQUAD 1								
Keratitis sicca	0	0	0	0	0	0	0	1
Total cataract	0	2	3	3	0	1	1	0
Diffuse anterior capsular cataract	0	0	0	0	0	0	3	3
SQUAD 2								
Total cataract	0	2	0	0	0	0	0	0
Focal lent. cat.	1	0	0	0	0	1	0	0
Dif. anter. capsule cataract	0	0	0	0	0	0	2	3
SQUAD 3								
Keratitis sicca	0	0	0	0	0	0	1	1
Focal nuclear cataract	0	0	0	0	0	0	1	0
Total ant. caps. cataract/dose group	0/15	0/15	0/15	0/15	0/15	0/15	5/15	6/15
Rt. eye or bilat.							3/15	2/15
Total cataracts, all types/dose gr.	1/15	4/15	3/15	3/15	0/15	2/15	7/15	6/15
Total keratitis sicca/dose group	0/15	0/15	0/15	0/15	0/15	0/15	2/15	2/15

1 Data taken from Table 3 of study report

By Day 20 of exposure, various types of cataracts were observed among all dose groups and squads. A slight increase in all types of cataracts was observed at high dose. Most cataracts were noted only in the left eye and may have resulted from ocular trauma during orbital sinus bleeding at Day 14. Diffuse anterior capsular cataracts were observed only in high dose animals of Squads 1 and 2, sometimes in the right or both eyes (total of 2 males and 3 females affected). Keratitis sicca was noted in 2 males and 2 females at high dose and may have been due to eye irritation from contact with the test material dust. Iritis, congestion of the iris or ciliary body, pthisis bulba, subluxation of the lens, panopthalmitis and focal retinochoroidal degeneration were observed as single incidences among control, low and mid dose squads and did not appear to be treatment-related.

Body Weights: Weekly body weight gains during the observation period are shown below in Table 5:

TABLE 5: BODY WEIGHT GAIN, GRAMS[1]

PNP:		0 mg/M^3	1 mg/M^3	5 mg/M^3	30 mg/M^3
Week 1	♂	49.1	52.1	56.0	53.4
	♀	34.4	26.5*	26.7*	27.4*
Week 2	♂	41.4	48.8	51.1*	42.8
	♀	22.1	19.9	25.4	22.6
Week 3	♂	29.9	33.0	28.1	38.2
	♀	13.7	15.6	11.9	12.0
Week 4	♂	21.5	19.7	31.8*	24.1
	♀	11.9	10.3	14.6	10.1
Wks 0 - 4	♂	141.8	154.1 (+8.7%)[2]	167.0* (+18%)	158.5 (+12%)
	♀	82.1	72.2 (-12%)	78.6 (-4.3%)	72.1 (-12%)

1 Data taken from Table 5B of study
2 % of controls

After one week of treatment, body weight gain of all treated females was about 23% lower than controls (statistically significant). Weight gain among treated females was comparable to controls during all subsequent weeks; the 12% lower weight gain (not statistically significant) in low and high dose females at the end of the study was therefore due primarily to the initial decrease. Since there was no dose-response, the decrease did not persist and was only noted in females and not males, it was not considered treatment related. Statistically significant decreases in mean body weight of about 5% compared to controls were noted in all treated groups at the end of Week 1. At termination, all dose groups showed approximately 5% lower mean

statistically significant reduction when compared to controls.

There were no treatment-related effects on mean body weights or mean body weight gain in males. Statistically significant increases in body weight were observed in the high dose at Weeks 3 and 4 and in mid dose at Week 4 (%) but were not considered treatment-related effects.

Hematology and Clinical Chemistry:

Blood was collected from 10 animals at termination for hematological analysis (including differential leukocyte count) and clinical chemistry analysis from all animals (fasted during 6hr exposure). In addition, all animals were bled at Day 14 of the study for hemoglobin and methemoglobin analysis. The CHECKED (X) parameters were examined.

Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count

Blood clotting measurements:

	(Thromboplastin time)
X	(Clotting time)
	(Prothrombin time)

* Required for subchronic and chronic studies

Results - At Day 14, no significant treatment-related effects on hemoglobin or methemoglobin concentrations were observed. Sporadic incidences of slightly increased methemoglobin were noted in the mid-dose group and slightly increased hemoglobin in the low dose group but these did not appear to be related to treatment since the effects were slight and there was no dose-response. Mean hemoglobin concentration was within the historical control range for all mean values.

TABLE 6: HEMATOLOGY PARAMETERS AT DAY 28 (END OF EXPOSURE PERIOD)[1]

PARAMETER	0 mg/M^3		1 mg/M^3		5 mg/M^3		30 mg/M^3	
	♂	♀	♂	♀	♂	♀	♂	♀
HGB, Gm/Dl	15.9	15.4	15.9	15.6	16.2	16.0	16.8*	16.0
HCT, %	47.9	45.7	48.5	46.2	48.5	47.1	51.3*	47.5
Platelet, Th/μL	892	1030	1100*	1096	1067*	975	975	1051
Clot. time, sec	665	105	111	89	102	86	94	91

1 Data taken from Table 6B of study report.
* $p < 0.05$

At Day 28, there were also no apparent treatment-related effects on measured blood parameter. Mean hemoglobin and hematocrit showed slight but statistically significant increases in high dose males but not females. The mean hematocrit of high dose males was slightly elevated above historical controls (51.1%, high range). Although TB-I agrees with the study authors that this was probably not a significant treatment-related effect, since hemoglobin is also slightly elevated it is possible that this represents a slight dehydration or stress effect among the high dose males.

Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase*^	X	Albumin/Globulin ratio
X	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
^ Not required for subchronic studies

Results - There were no apparent treatment-related effects on clinical chemistry parameters observed at any dose level in this study. Statistically significant elevation of total bilirubin and slight increase in BUN in low dose females did not appear to be biologically significant or treatment-related. All mean values were within historical control range.

Sacrifice and Pathology:

Necropsy: Animals were sacrificed on Day 29 by exsanguination under pentobarbital anesthesia. Organs were removed and examined for grossly visible lesions before processing for histopathological examination.

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta*	XX	Brain*,
X	Salivary glands*	XX	Heart*	X	Periph. nerve*#
X	Esophagus*	X	Bone marrow*	X	Spinal cord*#
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen	X	Eyes *#
X	Jejunum*	X	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
X	Colon*	X	Urinary bladder*	X	Mammary gland*#
	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver *	X	Epididymides	X	Thyroids*
	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle	X	Bone*#
Respiratory		X	Ovaries*	X	Skeletal muscle*#
X	Trachea*	X	Uterus*	X	Skin*#
XX	Lung*			X	All gross lesions/ masses*
X	Nasal turbinates				
	Larynx				
	Pharynx				

* Required for subchronic and chronic studies.
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
Organ weight required in subchronic and chronic studies.

Results - No grossly visible treatment-related effects were observed at necropsy.

Organ Weights: Selected absolute and relative organ weight values are presented below in Table 7:

TABLE 7: ABSOLUTE AND RELATIVE ORGAN WEIGHTS[1]

		0 mg/M^3		1 mg/M^3		5 mg/M^3		30 mg/M^3	
		♂	♀	♂	♀	♂	♀	♂	♀
Liver	Abs	8.50	6.44	8.82	6.02*	9.00	6.10	8.87	5.96*
	Rel	2.772	2.964	2.792	2.898	2.762	2.878	2.721	2.889
Lung	Abs	1.231	1.052	1.228	1.013	1.306	1.018	1.306	1.057
	Rel	0.4027	0.4836	0.3899	0.4877	0.4015	0.4809	0.4043	0.5126*
Heart	Abs	1.008	0.798	1.004	0.718	1.024	0.716	0.987	0.718
	Rel	0.3300	0.3656	0.3194	0.3456	0.3148	0.3373	0.3030	0.3480
Kidneys	Abs	2.335	1.668	2.409	1.581	2.463	1.561	2.449	1.552
	Rel	0.7634	0.7668	0.7628	0.7608	0.7567	0.7370	0.7517	0.7531

1 Data taken from Tables 10A and 10B of study report
* $p < 0.05$

Results - No significant treatment-related effects on organ weights were observed. Slight but statistically significant decreases in liver weight (about 7.5%) in low and high-dose females were observed but relative organ weights were not significant. Relative lung weight was slightly increased in high dose females (about 6%) but not males and did not appear to be treatment-related. No correlating gross or histopathologic effects were noted for any organ.

Histopathology: Organs examined for histopathology were fixed in 10% formalin solution except for the eyes and testes with epididymis (fixed in 10% Bouin's fluid, then 70% ethanol). Tissues were dehydrated in graded ethanol series, embedded in plastic and 5 μm sections stained with hematoxylin and eosin were examined using light microscopy.

Results - No apparent treatment-related histopathological lesions were observed. Appearance of dark foci in the thymus of two high-dose females was not considered treatment-related.

DISCUSSION:

TB-I agreed with the study author's conclusions that there were no apparent systemic, treatment-related effects observed in this study in rats exposed to doses up to 30 mg/M^3 of paranitrophenol. All treated females showed body weight decrease after Week 1 but there was no dose response and subsequent weight gain was comparable to controls. Minor effects on organ weights may have been in part due to the overall decrease in weight gain in the controls. Slight increases in hemoglobin and hematocrit in high dose males were not considered of biological significance.

However, TB-I considers the incidences of keratitis sicca to be a possible local irritation effect from the presence of paranitrophenol dust. The reason for the appearance of a few anterior capsular cataracts in the right eyes among high dose animals is not clear. The relatively high incidence of cataracts in young animals was unexpected. It is possible that some animals were bled from the right eye or both eyes at Day 14, or that unidentified environmental factors contributed to their formation. TB-I agreed with the study author that the increased incidence of anterior capsular cataracts was probably not treatment-related, since the animals showed no other systemic, treatment-related effects and were treated only for 20 exposures.

This study did not satisfy the intent of an acute inhalation study and an LC_{50} was not determined. The compound was not tested up to a limit dose and particle size was larger than required by the EPA Interim Guidelines on inhalation toxicology studies (at least 25% of particles at or below 4 μM MMAD). In addition, information on the purity of the test

than required by the EPA Interim Guidelines on inhalation toxicology studies (at least 25% of particles at or below 4 μM MMAD). In addition, information on the purity of the test material was not provided in this report.

LD_{50}: not determined in this study

Study Deficiencies: Particle size (MMAD) above 5 μm, clearly treatment-related toxicity not observed at any dose, oxygen concentration of air not mentioned, duration of study 4 weeks instead of single dose, purity of test compound not provided.

Classification: Unacceptable for 81-3; Supplementary for 82-4 (not upgradable).